UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

IN RE COLUMBIA UNIVERSITY PATENT LITIGATION	MDL No. 1592 (MLW) This Document Relates To All Actions

REBUTTAL EXPERT REPORT OF PROFESSOR FRANCIS H. RUDDLE SUBMITTED ON BEHALF OF THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

I. Introduction

I have reviewed the expert reports of Drs. Bruce J. Dolnick, Rodney E. Kellems and Harvey F. Lodish (collectively, the "plaintiffs' reports"), including the relevant references cited therein. Each of these reports recites a legal test for determining whether the claims of the '275 patent are invalid for double patenting. Dolnick ¶ 15; Kellems ¶ 46; Lodish ¶ 115. I do not believe that there is a meaningful difference between these standards for double patenting and the standard recited in my initial report. To the extent that there is any difference, however, none of my opinions would change if I were to apply the standards as phrased in their reports. Claims 3, 5-14, and 16-19 of the '275 patent are not invalid for double patenting in light of any claim of the original Axel patents, either alone or in combination with any other claim of the original Axel patents, or in combination with any knowledge in the art of the '275 patent as of February 1980.

I should also note at the outset that Dr. Dolnick's report states that "I have been informed that I can consider whether one of ordinary skill in the art as of February 25, 1980 would have inherently understood an element of a claim in the '275 patent to be present in a claim in a prior Axel patent even though that element is not explicitly mentioned in the claim of the prior Axel Patent." Dolnick ¶ 15. I have applied a different standard in the analysis in this rebuttal report. Specifically, I have been asked to consider whether a limitation is "inherent" regardless of whether it would be recognized as such by a person of skill in the art as of February 1980. As discussed below, it is my opinion that the plaintiffs' reports have not presented evidence that the presence of the "glycoprotein of interest" in claim 19 of the '275 patent, or the presence of amplified DNA II stably incorporated into the chromosomal DNA of the host CHO cell in claims 3,

5-14, and 16-19 of the '275 patent, are inherent in any of the claims of the original Axel patents.

Below is a response to what I consider to be the major relevant statements made in plaintiffs' reports. I reserve the right to supplement my analysis based on statements made in any rebuttal reports submitted by Drs. Dolnick, Kellems and Lodish. In addition, I reserve the right to provide a particularized analysis of the references cited in the reports and the statements made in the reports to the extent necessary to respond to specific issues that may become relevant.

II. Glycosylation of Foreign Polypeptides

The reports of Drs. Dolnick, Kellems and Lodish make two primary arguments about glycosylation. First, they argue that the "normal mechanisms" of action of a eukaryotic host cell necessarily result in the glycosylation of a foreign polypeptide that ordinarily would be gylcosylated in its natural environment. *See*, *e.g.*, Dolnick ¶¶ 457, 484; Kellems ¶ 204. Second, they argue that it would be obvious to persons of ordinary skill in the art in February 1980 that they could construct a eukaryotic host cell that is competent to glycosylate a foreign polypeptide and culture it under appropriate conditions so as to obtain the glycosylated foreign polypeptide. *See*, *e.g.*, Dolnick ¶ 459; Kellems ¶ 31; Lodish ¶¶ 142, 159. I will respond to these arguments in turn.

It is important to emphasize that merely because a eukaryotic host cell (such as a CHO cell) contains a gene for a foreign polypeptide that may be glycosylated does not mean that the host cell will necessarily glycosylate that foreign polypeptide. There are multiple reasons for this. First, not all host cells are competent to perform a particular type of glycosylation. For example, there are types of CHO cells that are incapable of

performing certain major glycosylations.[1] Therefore, if a foreign polypeptide normally receives a type of glycosylation that the host cell is incapable of performing, the foreign polypeptide either will not be glycosylated at all or will not be glycosylated appropriately. Second, even if a cell is competent to perform a particular type of glycosylation, it might fail to do so.[2] Indeed, this is a risk not just for glycosylation but for other post-translational modifications that might be required to make the "glycoprotein of interest" of claim 19 of the '275 patent.[3] For example, even if a cell is competent to perform a particular type of post-translational modification, the appropriate culture conditions necessary to facilitate that type of post-translational modification may not exist.[4] The environment in which a cultured host cell exists is different from its

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[1] *See, e.g.,* Kozarsky, K. *et al.*, "Use Of A Mutant Cell Line To Study the Kinetics and Function of O-linked Glycosylation of Low Density Lipoprotein Receptors," *Proc. Nat. Acad. Sci. USA* 85:4335-4339 (1988).

[2] McCormick, *et al.,* "Inducible Expression of Amplified Human Beta Interferon Genes in CHO Cells," *Mol. Cell. Biol.* 4(1):166-172 (1984).

[3] Kaufman, R.J. *et al.*, "Expression, Purification, and Characterization of Recombinant γ-Carboxylated Factor IV Synthesized in Chinese Hamster Ovary Cells," *J. Biol. Chem.* 261(21):9622-9628 (1986).

[4] Kaufman, R.J. *et al.*, "Expression, Purification, and Characterization of Recombinant γ-Carboxylated Factor IV Synthesized in Chinese Hamster Ovary Cells," *J. Biol. Chem.* 261(21):9622-9628 (1986); Kaufman, R.J. *et al.*, "Synthesis, Processing, and Secretion of Recombinant Human Factor VIII Expressed in Mammalian Cells," *J. Biol. Chem.* 263(13):6352-6362 (1988); Goochee, C. F. *et al.*, "Environmental Effects on Protein Glycosylation," *Biotechnology* 8:421-424 (1990); Andersen, D.C. *et al.*, "The Effect of Cell-culture Conditions on the Oligosaccharide Structures of Secreted Glycoproteins," *Cur. Opin. Biotechnol.* 5:546-549 (1994); Jenkins, N., *et al*, "Glycosylation of Recombinant proteins: Problems and Prospects," *Enzyme Microb. Technol.*, 16:354-364 (1994). *See generally,* Stanley, P., "Glycosylation Engineering," *Glycobiology*, 2(2):99-107 (1992); Goochee, C.F. *et al*, "The Oligosaccharides of Glycoproteins: Bioprocess Factors Affecting Oligosaccharide Structure and Their Effect on Glycoprotein Properties," *Biotechnology* 9:1347-1355 (1991); Goochee, C.F. *et al*, "The Oligosaccharides of Glycoproteins: Factors Affecting Their Synthesis and Their

natural environment. Removing a cell from its natural environment may rob it of the materials and conditions necessary to allow it to perform a given post-translational modification. Thus, certain combinations of host cell, foreign DNA, and cell culture conditions may produce a polypeptide with deficient or missing post-translational modifications (including glycosylation). Thus, no claim of the original Axel patents recites an invention that necessarily leads to the production of the "glycoprotein of interest" of claim 19 of the '275 patent under normal conditions.

In addition, a person of any level of skill in the art at the time of the invention would not have considered the presence of the "glycoprotein of interest" of claim 19 to be an obvious variant of the claims in the original Axel patents. I discuss this issue at length in my initial report. *See* Ruddle, pp. 9-20.

Drs. Dolnick, Kellems, and Lodish contend that claim 19 of the '275 patent would be obvious in light of the original Axel patents. They cite a large number of references in their reports to support their position, but they do not explain the relevance of most of them. This necessarily limits my ability to respond in detail to their reports. Based upon my review of their reports and the relevant references cited in the reports, I can find nothing that calls into question the conclusions I reached in my initial report regarding claim 19 of the '275 patent: It is not obvious in light of the claims of the original Axel patents. There are some specific references and arguments that Drs. Dolnick, Kellems and Lodish make that I believe are worth discussing in more detail.

In January 1980, Bert O'Malley's laboratory at Baylor published a paper reporting that they had transformed a mouse cell with the gene for chicken ovalbumin (a

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Influence on Glycoprotein Properties," in *Frontiers in Bioprocessing II* 199-240 (Todd, P. *et al* eds. 1990).

polypeptide that, in nature, can be glycosylated).[5] However, a person of skill in the art would not view glycosylation of a foreign polypeptide as obvious in light of this reference. The O'Malley laboratory used two tests to identify the foreign polypeptide produced by the mouse host cell. First, they ran an immunoassay to determine if the foreign polypeptide interacted with an anti-ovalbumin antibody. Antibodies can recognize certain structures on a molecule and attach to those structures. Second, they compared the size of the foreign polypeptides to a crystalline form of the naturally occurring ovalbumin. Neither of these tests established that the foreign polypeptides that had been produced were glycosylated. To the contrary, the comparison of size test showed that there was a difference in size between the naturally occurring ovalbumin and the foreign polypeptide. The reference suggests that the difference in size "should not be interpreted as a consequence of differing peptide length . . . . Rather, the small differences [in size] probably represent dissimilar or incomplete posttranslational modifications of the polypeptides." pp. 246. None of the tests that would have actually established whether the foreign polypeptide had been glycosylated was performed. The size of the foreign polypeptide could have been a function of improper translation of the ovalbumin gene. Simply put, a person of skill in February 1980 would not view this reference as making obvious that glycosylation of a foreign polypeptide will ordinarily occur in a mammalian cell.

However, even if one were to assume that the O'Malley laboratory established that glycosylation occurred in this particular system, a person of any level of skill in February 1980 would still doubt that a host cell would perform appropriate glycosylation

[5] Lai, E. *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. USA* 77(1):244-248 (1980).

and other post-translational modifications. In fact, the O'Malley laboratory's reference to "dissimilar or incomplete posttranslational modifications" would only serve to reinforce these doubts.

The plaintiffs' reports also cite a group of references that relate to experiments monitoring the results of viral infection of eukaryotic cells.[6] Viruses are parasites that rely on other organisms to live and propogate. A virus enters a cell and then modifies or rearranges cellular mechanisms for a particular purpose. One of the things a virus can instruct a cell to do is produce particular glycosylated proteins. The plaintiffs' reports appear to suggest that the fact that eukaryotic cells produce viral glycoproteins when infected establishes that they will glycosylate a polypeptide made under the direction of foreign DNA inserted into the cell via a DNA-mediated gene transfer system. *See, e.g.*, Lodish ¶¶ 142, 145, 159. It is important to emphasize that viral infection of a cell is a common and standard event in nature. For millions of years, organisms have been exposed to viruses and mounted responses specifically against them. Because of this, there is a distinction between experiments involving a naturally occurring event (the

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[6] *See, e.g.*, Kornfeld, S. *et al.*, "The Synthesis of Complex-type Oligosaccharides. II: Characterization of the Processing Intermediates in the Synthesis of the Complex Oligosaccharide Units of the Vesicular Stomatitis Virus G Protein," *J. Biol. Chem.* 253(21):7771-7778 (1978); Li, E. *et al.*, "Biosynthesis of Lipid-linked Oligosaccharides. Isolation and Structure of a Second Lipid-linked Oligosaccharide in Chinese Hamster Ovary Cells," *J. Biol. Chem.* 254(8):2754-8 (1979); Li, E. *et al.*, "The Synthesis of Complex-type Oligosaccharides. I. Structure of the Lipid-linked Oligosaccharide Precursor of the Complex-type Oligosaccharides of the Vesicular Stomatitis Virus G Protein," *J. Biol. Chem.* 253(21):7762-70 (1978); Tabas, I. *et al.*, "Processing of High Mannose Oligosaccharides to Form Complex Type Oligosaccharides on the Newly Synthesized Polypeptides of the Vesicular Stomatitis Virus G and the IgG Heavy Chain," *J. Biol. Chem.* 253(3):716-22 (1978); Tabas, I. *et al.*, "The Synthesis of Complex-type Oligosaccharides: III. Identification of an a D-Mannosidase Activity Involved in a Late Stage of Processing of Complex-Type Oligosaccarides," *J. Biol. Chem.* 253(21):7779-7786 (1978).

infection of a cell with a natural virus) and a non-naturally occurring event (such as DNA-mediated gene transfer, which involves genetic engineering). A person of any level of skill in the art in 1980 would not have considered natural viral-infection experiments as indicative of how a cell's apparatus for post-translational modification would respond to the genetic engineering inventions that are the basis of the Axel patents.[7]

Even if the reported experiments involving viral infection established that a eukaryotic cell will glycosylate a foreign polypeptide, they do not establish that a eukaryotic cell (such as a CHO cell) would be able to execute the appropriate glycosylation and other post-translational modifications sufficient to produce the "glycoprotein of interest" of claim 19 of the '275 patent. *See, e.g.*, '275 patent, 2:38-3:20; 7:31-58. In fact, before February 1980 workers performed a series of viral infection experiments not addressed by the plaintiffs' reports that suggest just the opposite. In these experiments, different types of eukaryotic cells were each infected with the same

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[7] For similar reasons, a person of any level of skill in the art in 1980 would not have considered experiments involving cell-free systems as indicative of what would occur in a DNA-mediated gene transfer system. *See, e.g.,* Rothman, J.E. *et al.*, "Synchronised Transmembrane Insertion and Glycosylation of a Nascent Membrane Protein," *Nature* 269:775-780 (1977); Tucker, P. *et al.*, "De Novo Synthesis and Glycosylation of MOPC-46B Mouse Immunoglobulin Light Chain in Cell-free Extracts," *J. Biol. Chem.* 252(13):4474-86 (1977). In a cell-free system, a researcher takes extracts from cells and adds a specific mRNA transcript to the extracts. The researcher then monitors how the enzymes in the cell extracts act on the mRNA transcript, for example by translating it into a polypeptide. A cell free system necessarily lacks the controls and regulatory structures that exist in an intact cell. Therefore, the fact that a polypeptide is treated in a particular way in a cell-free system does not mean the polypeptide will be treated the same way in an intact cell. In addition, even if one assumed that data from cell-free systems was relevant to the analysis of the obviousness of claim 19 of the '275 patent, a person of skill in the art in February 1980 would view the data from these experiments as casting doubt on whether a host cell could appropriately execute the post-translational modifications that occur on a foreign polypeptide in its natural environment. Tucker, P. *et al.*, "De Novo Synthesis and Glycosylation of MOPC-46B Mouse Immunoglobulin Light Chain in Cell-free Extracts," *J. Biol. Chem.* 252(13):4474-86 (1977).

virus. The same viral glycoprotein produced by this virus in each cell type was then analyzed for similarities and differences. These experiments indicated that there were differences in glycosylation performed by the different cell types on the same viral polypeptide.[8]

Plaintiffs' reports also reference research describing certain types of glycosylation performed on native polypeptides in various eukaryotic cells, specifically CHO cells.[9] The plaintiffs' reports appear to suggest that these experimental results showing that CHO cells have the capacity to perform specific types of glycosylation on native polypeptides in certain contexts establish that it is obvious that a host cell would glycosylate a foreign polypeptide. *See, e.g.*, Lodish ¶¶ 142, 145, 159. The fact that CHO cells may attach certain standard sugar structures in glycosylation reactions involving native polypeptides does not establish how that host cell would treat a foreign polypeptide. The relevant question is not whether a given host cell can glycosylate native proteins, or even whether

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[8] Etchison, J. R. *et al.*, "Carbohydrate Composition of the Membrane Glycoprotein of Vesicular Stomatitis Virus Grown in Four Mammalian Cell Lines," *Proc. Nat. Acad. Sci. USA* 71(10):4011-4014 (1974); Moyer, S., *et al.*, "Oligosaccharide Moieties of the Glycoprotein of Vesicular Stomatitis Virus," *J. Virol.* 18(1):167-175 (1976); Strauss, J. *et al*, "Carbohydrate Content of the Membrane Protein of Sindbis Virus," *Mol. Biol.* 47:437-448 (1970); McSharry, J. *et al*, "Biological Properties of the VSV Glycoprotein," *Virology* 84:183-188 (1978); *see also* Burge, B.W. *et al.*, "Comparison of Membrane Protein Glycopeptides of Sindbis Virus and Vesicular Stomatitis Virus," *J. Virol.* 6(2):176-182 (1970).

[9] *See, e.g.,* Li, E. *et al.,* "Structural Studies of the Major High Mannose Oligosaccharide Units From Chinese Hamster Ovary Cell Glycoproteins," *J. Biol. Chem.* 254(5): 1600-5 (1979); Narasimhan, S. *et al.*, "Control of Glycoproteins Synthesis: Lectin-resistant Mutant Containing Only One of Two Distinct N-Acetyiglucosaminyltransferase Activities Present in Wild Type Chinese Hamster Ovary Cells," *J. Biol. Chem.* 252(11):3926-3933 (1977). *See also,* Chu, F. *et al.*, "The Effect of Carbohydrate Depletion on the properties of Yeast External Invertase," *J. Biol. Chem.* 253(24):8691-8693 (1978).

it can add certain glycoprotein structures. Rather, the relevant question is whether as of February 1980 a person of ordinary skill in the art would consider it obvious that a eukaryotic host cell (or in specific a CHO cell) possesses and can effectively use the enzymes and other structures sufficient to post-translationally process a foreign polypeptide effectively. There simply was not enough data on (among other things) signaling structures, the effects of culturing, and species and cell type differences in post-translational modification for a person of any level of skill in the art to consider this obvious.

It is also worth noting that plaintiffs' reports cite references that provide extremely detailed analysis of carbohydrate structures added by eukaryotic cells (and mutants of such cells). Persons of ordinary skill in the art in February 1980 would not have had a sufficient understanding of how to apply the detailed conclusions of these references to address the problems solved by the '275 patent. It was many years after February 1980 before ordinary researchers in the field of recombinant DNA technology began deploying the sophisticated analytical tools described in these references. Persons of ordinary skill in the art of recombinant DNA technology in February 1980 would have had a basic understanding of glycoprotein biochemistry. For example, they would have understood that there was evidence of a role played by signaling structures. They would also have understood the potential for species and cell-type differences in glycosylation. However, persons of ordinary skill in the art of the '275 patent in February 1980 would have lacked

the intense focus on glycoprotein biochemistry necessary to truly understand and apply fully the information in the references cited in plaintiffs' reports.[10]

The plaintiffs' reports suggest that by February 1980, researchers were beginning to collect data indicating that a eukaryotic cell would accurately transcribe and translate foreign DNA in a co-transformation system involving both a marker DNA and a DNA of interest. *See, e.g.*, Dolnick ¶ 35; Lodish ¶ 62.[11] The papers cited in the plaintiffs' reports represent the early stages of detailed study on the structure of RNA and polypeptides produced from foreign genes. A person of ordinary skill in the art in February 1980 would not have viewed this data as making it obvious that proper transcription and translation would always occur. In addition, merely assuming that a foreign gene is transcribed and translated properly in a particular system would not have made it obvious in February 1980 that the host cell would glycosylate the foreign polypeptide. Glycosylation is not just a function of the polypeptide's sequence of amino acids; it is a function of how the host cell interacts with the polypeptide. *See* Ruddle, pp. 9-12.

Finally, Drs. Dolnick, Kellems and Lodish each assert that by February 1980, persons of ordinary skill in the art understood that interferon, clotting factors and antibodies were glycoproteins. *See, e.g.*, Dolnick ¶¶ 145-147, 155-158, 163-166; Kellems ¶ 30; Lodish ¶¶ 76, 78, 143. Interferon, clotting factors, enzymes and antibodies

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[10] Even though I view a number of the references cited in plaintiffs' report as outside the skill and understanding of a person of ordinary skill in the art, I still consider them as part of the art for the purposes of my analysis.

[11] Notably, it is not clear from all the references plaintiffs' reports cite that the host cell is functioning properly. *See, e.g.,* Mantei, N. *et al.,* "Rabbit β-globin mRNA Production in Mouse L Cells Transformed With Cloned Rabbit β-globin Chromosomal DNA," *Nature* 281:40-46 (1979); Mulligan, R.C. *et al.*, "Synthesis of Rabbit β-Globin in Cultured Monkey Kidney Cells Following Infection with a SV40 β-Globin Recombinant Genome," *Nature* 277:108-114 (1979).

encompass large classes of molecules that, in February 1980, were not fully known or analyzed, and therefore a person of ordinary skill in the art in February 1980 would view them as potentially including both glycosylated and non-glycosylated molecules. For example, workers in the field understood that there were both glycosylated and non-glycosylated enzymes.[12] Workers also understood that non-glycosylated interferon and non-glycosylated antibody still retained their identity as interferons and antibody.[13] Even after February 1980, workers in the field did not view glycosylation as necessarily defining the identity of the molecule.[14] Thus, a person of skill in the art as of February 1980, viewing a reference to DNA encoding "interferon," "clotting factor," an "antibody," or an "enzyme," would understand these terms to refer to DNA that encoded polypeptides that can be glycosylated, but may not be glycosylated in all contexts.[15] Thus, even after February 1980, when a gene for a type of interferon was inserted into a prokaryotic cell

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[12] Kauffman, D. *et al.,* "The Relationship of Flow Rate to Glycosylation of Human Parotid Amylase," *Archs. oral Biol.* 19:597-599 (1974); Baynes, J.W. *et al.*, "Effect of Glycosylation on the *in Vivo* Circulating Half-life of Ribonuclease," *J. Biol. Chem.* 251(19) 6016-6024 (1976).

[13] Hickman, S. *et al.*, "Effect of Tunicamycin on IgM, IgA, and IgG Secretion By Mouse Plasmacytoma Cells," *J. Immunol.* 121(3):990-6 (1978); Fujisawa, J., "Nonglycosylated Mouse L Cell Interferon Produced by the Action of Tunicamycin," *J. Biol. Chem.* 253(24):8677-79 (1978).

[14] Lodish, H. F., "Post-translational Modification of Proteins," *Enzyme Microb. Technol.* 3:178-188 (1981).

[15] Plaintiffs' reports observe that the insertion of a foreign DNA that encoded a polypeptide that in nature may be glycosylated into a eukaryotic cell was obvious in February 1980, citing, for example, Taniguchi, T. *et al.,* "Construction and Identification of a Bacterial Plasmid Containing the Human Fibroblast Interferon Gene Sequence," *Proc. Japan. Acad.* 55 Ser. B:464-69 (1979). However, this does not make obvious that a eukaryotic host cell (in specific a CHO cell) would glycosylate the foreign polypeptide encoded by the gene.

and expressed, researchers still referred to the polypeptide product as interferon even though it was not glycosylated.[16]

It is important to emphasize again that post-translational modifications (and glycosylation in specific) are complex. In February 1980, workers were aware that just as the total absence of a specific post-translational modification can have a significant impact on the usefulness of a molecule, an improper or aberrant post-translational modification might also have a significant impact.[17] In February 1980, it simply would not have been obvious to a person of skill in the art (whether ordinary or extraordinary) that a host cell could perform the appropriate post-translational modifications to produce the "glycoprotein of interest" of claim 19 of the '275 patent. In addition, as discussed in my original report, no claim of the original Axel patents recites the production of a "glycoprotein of interest." Ruddle, pp. 16-20.

III. Use of Chinese Hamster Ovary Cells

Drs. Dolnick, Kellems and Lodish state that the use of a CHO cell to practice the claims of the '275 patent would have been obvious. *See, e.g.*, Dolnick ¶¶ 24-25; Kellems ¶¶ 35; Lodish ¶¶ 64, 104, 145-148. However, there is a difference between whether a worker might think it appropriate to experiment with the use of a DNA-mediated gene transfer system in a CHO cell, and whether it would be obvious to a person of ordinary skill in the art as of February 1980 that a CHO cell could be used successfully to practice

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[16] Goeddel, D. V. *et al.*, "Human Leukocyte Interferon Produced by *E. coli* Is Biologically Active," *Nature* 287:411-416 (1980); *see also* Boss, M.A. *et al.*, "Assembly of Functional Antibodies from Immunoglobulin Heavy and Light Chains Synthesized in *E coli*," *Nucleic Acids Research* 12(9):3791-3806 (1984).

[17] Ruddle, nn. 14-15; *see generally,* Winterburn, P. *et al.*, "The Significance of Glycosylated Proteins," *Nature* 236:147-151 (1972).

the claims of the '275 patent. As discussed in my initial report, there simply was not the critical mass of information on the parameters necessary for successful DNA-mediated gene transfer to give a person of ordinary skill in the art in February 1980 confidence that CHO cells could be used successfully to practice the claims of the '275 patent.

In support of their assertions, plaintiffs' reports focus on the fact that in another system for transfer of genetic material, chromosome-mediated gene transfer, workers had used Chinese Hamster cells.[18] However, chromosome-mediated gene transfer, which involves the insertion of chromosomes into a host cell, is not comparable to DNA-mediated gene transfer (the technology implicated by the Axel patents). This is due to the structural differences between chromosomes and individual pieces of DNA. Chromosomes are made up of tightly compacted DNA and proteins. In February 1980, there was an understanding that the structure of genetic material inserted into a host cell could influence its fate in the cell, in particular its susceptibility to compounds inside the cell.[19] For example, workers before February 1980 recognized that it was much more difficult to perform successful DNA-mediated gene transfer experiments than

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[18] *See* Wullems, G.J. *et al.*, "Incorporation of Isolated Chromosomes and Induction of Hypoxanthine Phosphoribosyltransferase in Chinese Hamster Ovary Cells," *Somatic Cell Genet.* 1(2):137-152 (1975); Wullems, G.J. *et al.*, "Expression of Human Hypoxanthine Phosphoribosyl Transferase in Chinese Hamster Cells Treated with Isolated Human Chromosomes," *Somatic Cell Genet.* 2(2):155-64 (1976). *See also* Kuroda, Y. *et al*, "Isolation and Characterization of Variant Clones of Chinese Hamster Cells After Treatment With Irradiated 5-Iodouridine," *Mutat. Res.* 33:285-298 (1975).

[19] For similar reasons, persons of skill in the art would likewise not view cell hybrid or fusion experiments involving Chinese hamster cells as indicative of what would occur in a DNA-mediated gene transfer experiment.

chromosome-mediated gene transfer experiments.[20] The fact that something occurred in a chromosome-mediated transfer system does not mean that it would necessarily occur in a DNA-mediated transfer system.[21] Therefore, just because it was possible to use Chinese Hamster cells in chromosome-mediated gene transfer does not mean that CHO cells could be used successfully to practice the '275 patent.[22]

Dr. Lodish states that the use of a CHO cell would have been obvious "because the processes by which proteins are transcribed and translated in cells are generally conserved among eukaryotic or mammalian cells and do not differ in any important respect from one eukaryotic or mammalian cell to another eukaryotic or mammalian cell." Lodish ¶ 104. This statement would have been accepted as a *hypothesis* in February 1980 for foreign genes inserted into a host cell via DNA-mediated transfer. However, there was not sufficient evidence available as of February 1980 for a person of ordinary skill in the art to conclude that it was obvious that this would be the case in all circumstances.

Plaintiffs' reports also emphasize that, by February 1980, there was a large amount of information known about CHO cells, and there were CHO cell lines available

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[20] *See generally,* Willecke, K., "Results and Prospects of Chromosomal Gene Transfer between Cultured Mammalian Cells," *Theor. Appl. Genet.* 52:97-104 (1978).

[21] My laboratory worked with Chinese Hamster cells in gene transfer experiments before February 1980. Because of the differences between the transfer systems used in these experiments, a person of ordinary skill in February 1980 would not have considered this work sufficient to make obvious that a Chinese hamster cell could be used to practice the '275 patent claims.

[22] It would also not have been obvious to a person of ordinary skill in February 1980 that simply because one type of cell derived from a given animal acted in a particular way, a different type of cell from the same animal would act in the same way.

for use in transformation experiments.[23] *See, e.g.*, Dolnick ¶¶ 24-25; Kellems ¶ 35; Lodish ¶¶ 64, 104, 145-148. Given the difficulty of DNA-mediated gene transfer, merely because a person of ordinary skill in the art in February 1980 would have had the tools or motivation to attempt DNA-mediated gene transfer in a CHO cell hardly means that such person would have considered it obvious that he or she would be successful in practicing the claims of the '275 patent.

An indication of the lack of certainty that DNA-mediated gene transfer would work in any type of host cell is the work of the Lawrence Chasin laboratory at Columbia. Dr. Chasin appeared to be contemplating the use of a CHO host cell for DNA-mediated gene transfer.[24] However, when his laboratory performed and reported transformation experiments, it focused on experiments in mouse cells, and reported an inability to transform CHO cells using DNA-mediated gene transfer.[25]

In February 1980, there were concerns about the reproducibility of experiments involving gene transfer with CHO cells. For example, the laboratory of Louis Siminovitch at the University of Toronto reported chromosome-mediated gene transfer experiments involving CHO host cells.[26] However, serious questions were raised about

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[23] In fact, my laboratory did work on characterizing the Chinese hamster genome in part because Chinese hamster cells were candidates for use in genetic experiments.

[24] Abstract of Grant Number 5R01GM022629-05, "Mitotic Recombination in Cultured Mammalian Cells," awarded to Chasin, L., Columbia University Fiscal Year 1979, Project Start 01-Sep-1978, Project End 31-Aug-1981.

[25] Graf, L.H. *et al.*, "Transformation of the Gene for Hypoxanthine Phosphoribosyltransferase," *Somatic Cell Genet* 5:1031-1044 (1979).

[26] Spandidos, D. A. *et al.*, "Linkage of Markers Controlling Consecutive Biochemical Steps in CHO Cells as Demonstrated by Chromosome Transfer," *Cell* 12:235-242 (1977); Spandidos, D. A. *et al.*, "Transfer of Codominant Markers by Isolated Metaphase

the reproducibility of this data. In fact, Dr. Siminovitch later disclaimed the accuracy of those experiments.[27]

For the reasons discussed above and in my original report, an individual of ordinary skill would consider as relevant data the reports on the use of Chinese hamster cells in different transfer systems, information known about CHO cells (e.g., their natural glycosylation patterns, or the status of amplified native DHFR), and the initial work of Drs. Lewis and Srinivasan on the use of CHO cells in DNA-mediated gene transfer (to the extent it was available).[28] However, a person of ordinary skill in February 1980 would not consider such data sufficient to make it obvious that the claims of the '275 patent could be successfully practiced in CHO cells.[29]

IV. Stable Incorporation

Drs. Dolnick, Kellems and Lodish all argue that the limitation that DNA II is stably incorporated into the chromosomal DNA of the host cell is obvious in light of the

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Chromosomes in Chinese Hamster Ovary Cells," *Proc. Natl. Acad. Sci. USA* 74(8):3480-3484 (1977).

[27] Lewis, W. H. *et al.*, "Parameters Governing the Transfer of the Genes for Thymidine Kinase and Dihydrofolate Reductase into Mouse Cells Using Metaphase Chromosomes or DNA," *Somatic Cell Genet.* 6(3):333-348 (1980).

[28] Indeed, if the work of Lewis and Srinivasan were available, it would suggest both that CHO cells may not be a desirable line to attempt to practice the '275 patent in and that DHFR may not be a desirable DNA II to use because it yielded unstable transformants. Lewis, W. H. *et al.*, "Parameters Governing the Transfer of the Genes for Thymidine Kinase and Dihydrofolate Reductase into Mouse Cells Using Metaphase Chromosomes or DNA, *Somatic Cell Genet.* 6(3):333-348 (1980); Srinivasan, P. R. *et al.*, "Transfer of the Dihydrofolate Reductase Gene into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," *Introduction of Macromolecules into Viable Mammalian Cells* 27-45 (1980).

[29] In fact, after February 1980, laboratories (including my own) were attempting to make DNA-mediated gene transfer an efficient system across cell-types. Loyter, A. *et al*, "Mechanisms of DNA Entry Into Mammalian Cells," *Exp Cell Res* 139: 223-234 (1982).

state of knowledge as of February 1980 and the claims of the original Axel patents. They also appear to argue that, in various claims of the original Axel patents, DNA II necessarily must be incorporated into the chromosomal DNA. For the reasons discussed in my initial report, I disagree with these statements. Ruddle, pp. 21-28. The lack of knowledge about the mechanism of action for amplification made it non-obvious that a person of skill in the art could obtain a host cell in which amplified DNA II is stably incorporated into the chromosomal DNA in light of the original Axel patents. In addition, it is not necessarily the case that amplified DNA II in the claims of the original Axel patents is present in the chromosomal DNA. Depending on the cell and environment in which it had been cultured, the normal location for amplified DNA may be non-stable and/or non-chromosomal.

Drs. Dolnick, Kellems and Lodish each make a number of assertions relating to stable incorporation of DNA II, and then cite a number of references, usually without explaining the relevance of those references. This makes it difficult to respond in detail to the reports. Despite this, after having reviewed the reports and the relevant references cited, I reaffirm the conclusions presented in my original report. However, there are some particular aspects of the reports that are worth discussing at greater length.

Plaintiffs' reports assert that if DNA I and DNA II are linked before insertion in a host cell, it would be obvious that they will both stably incorporate into the chromosomal DNA of the host cell. *See*, *e.g.*, Dolnick ¶ 28. In February 1980, there simply was not enough evidence available in the DNA-mediated cell transformation context for that

conclusion to be obvious.[30] Papers from before February 1980 dealing with the insertion of DNA I and DNA II into a host cell reported that it was unclear where the DNA was located.[31] In addition, it was not clear before February 1980 that large pieces of DNA would necessarily remain intact upon insertion into a host cell.[32] There was also

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[30] Substantially more data existed on chromosome-mediated gene transfer experiments, in part based on work in my laboratory. Despite the fact that workers had more data of chromosome-mediated gene transfer, a person of skill would not have considered it obvious that a DNA-mediated gene transfer experiment would follow the same exact patterns. Chromosome-mediated gene transfer involves genetic material that is fundamentally different in structure than DNA-mediated gene transfer. Chromosomes are highly compacted structures made up of DNA and proteins. A person of skill in the art in February 1980 would have recognized that the fundamental differences in structure of the genetic material would invariably impact how it interacted with a cell, in particular its susceptibility to compounds inside the cell. Thus, it was recognized in February 1980 that chromosome-mediated gene transfer was more reproducible than DNA-mediated gene transfer. *See generally* Willecke, K., "Results and Prospects of Chromosomal Gene Transfer between Cultured Mammalian Cells," *Theor. Appl. Genet.* 52:97-104 (1978).

[31] Mantei, N. *et al.,* "Rabbit β-globin mRNA Production in Mouse L Cells Transformed With Cloned Rabbit β-globin Chromosomal DNA," *Nature* 281:40-46 (1979), at 45 ("Previous experience suggests that the exogenous DNA is largely integrated into host cell sequences, but this point requires further investigation."); Huttner, K. *et al.*, "DNA-Mediated Gene Transfer of a Circular Plasmid into Murine Cells," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5820-5824 (1979); Breathnach, R. *et al.*, "Correct Splicing of a Chicken Ovalbumin Gene Transcript in Mouse L Cells," *Proc. Natl. Acad. Sci. USA* 77(2):740-744 (1980), at 743 ("The ovalbumin gene copies could be incorporated into host cell sequences, as previously suggested, or they could be associated with the carrier DNA used in the transformation to form some sort of 'double-minute-like' chromosomes."); *compare* Graf, L.H. *et al.*, "Transformation of the Gene for Hypoxanthine Phosphoribosyltransferase," *Somatic Cell Genet* 5:1031-1044 (1979), at 1042 ("The state of the assimilated hprt gene in the transformed mouse cell is not known."); Pellicer, A. *et al.*, "The Transfer and Stable Integration of the HSV Thymidine Kinase Gene into Mouse Cells," *Cell* 14:133-141 (1978), at 140 ("The evidence is compelling that integration has occurred. We have not, however, demonstrated chromosomal integration . . . ."); Weissmann, C., "Future Trends: Reversed Genetics," *TIBS* 3:N109-N111 (1978); Perucho *et al.,* "Genetic and Physical Linkage of Exogenous Sequences in Transformed Cells," *Cell* 22: 309-17 (1980).

[32] *See generally,* Lai, E., *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene," *Proc. Natl. Acad. Sci. USA* 77(1):244-248 (1980); Weissmann, C. *et al.*, "Expression of Cloned Viral and Chromosomal Plasmid-

evidence from both before and after February 1980 that stable transformation does not necessarily occur upon the successful insertion of genetic material into a host cell.[33]

Once foreign DNA I and foreign DNA II are amplified (which is required by claims 3, 5-14, and 16-19 of the '275 patent), the location of DNA I and DNA II would have been even less clear. This is because the mechanism of action for amplification was not clear in February 1980. Amplification is an aberrant event that reflects instability in the genetic makeup of a cell.[34] As discussed in my initial report, amplification can lead to extra-chromosomal and unstable amplified DNA.[35]

The above discussion makes clear that, in February 1980, it would not have been obvious that, when foreign DNA I and foreign DNA II (whether linked or unlinked for insertion) are amplified and/or foreign DNA I is stably incorporated into the chromosomal DNA, DNA II is stably incorporated in the chromosomal DNA. It was

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linked DNA in Cognate Host Cells," in *From Gene to Protein Information Transfer in Normal and Abnormal Cells* 99-132 (Russell *et al.* eds. 1979).

[33] *See generally,* Graf, L.H. *et al.*, "Transformation of the Gene for Hypoxanthine Phosphoribosyltransferase," *Somatic Cell Genet* 5:1031-1044 (1979); Perucho *et al.*, "Genetic and Physical Linkage of Exogenous Sequences in Transformed Cells," *Cell* 22: 309-17 (1980); Wigler, M. *et al.*, "Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells," *Cell* 11:223-232 (1977).

[34] There were multiple observations of endogenous amplification or possible endogenous amplification by February 1980. *See, e.g.,* Hildebrand, C.E. *et al.*, "A Cadmium-resistant Variant of the Chinese Hamster (CHO) Cell with Increased Metallothionein Induction Capacity," *Exp. Cell Res.* 124:237-246 (1979); Wahl, G. M. *et al.*, "Gene Amplification Causes Overproduction of the First Three Enzymes of UMP Synthesis in *N*-Phosphonacetyl-$_L$-aspartate-resistant Hamster Cells," *J. Biol. Chem.* 254(17):8679-8689 (1979); Schimke R. T. *et al.*, "Studies on the Amplification Of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.* 43:1297-1303 (1979).

[35] Ruddle, n. 26.

recognized in February 1980 that DNA can persist unstably and outside of the chromosomal DNA and that the method of action of amplification, especially amplification involving foreign DNA, was not known. Indeed, based on current evidence, this continues to be the case.[36] Thus, the fact that DNA I and DNA II are linked at one time and/or DNA I is stably incorporated into the chromosomal DNA does not mean that DNA II necessarily will be stable and incorporated into the chromosomal DNA.

Dr. Lodish's report appears to maintain that if amplified DNA II is not stably incorporated into the chromosomal DNA, it "would not be passed on to successive generations." *See, e.g.*, Lodish ¶¶ 112, 63. This was not obvious in February 1980, nor is it necessarily correct. When amplified DNA II is not stably incorporated into the chromosomal DNA of the host cell, then the offspring of the host cell may not receive equal amounts of the amplified DNA II. However, the amplified DNA II will not necessarily be "lost." It may be inherited by the daughter cells; it just might not be inherited in equal amounts. One daughter cell may contain most or all of the amplified DNA II, while another daughter cell might not contain any.[37] DNA II in the Axel patents is a marker DNA – it confers a property on the host cell that allows the host cell to be identified. For example, DNA II can confer the property of a certain level of resistance to a poison. (The administration of the poison is sometimes referred to as "selection pressure.") The more copies of DNA II that are within the host cell, the higher its level

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[36] Ruddle, n. 26.

[37] Kaufman, R. J., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-resistant Cells are Associated with Double Minute Chromosomes," *Proc Natl Acad Sci USA.* 76(11):5669-5673 (1979).

of resistance to the poison. Thus, the daughter cell that receives more copies of amplified DNA II may have extremely high resistance to the poison that DNA II confers resistance to, while the other may have limited or no resistance. Under selection pressure, the latter will die, while the former will live.

Dr. Lodish observes that "[a] person of ordinary skill in the art at the time of the invention would have known that the selection pressure in the claim [claim 54] . . . would permit the isolation of cells that have stably incorporated the foreign DNA into their genome. Thus, it would be obvious to one of ordinary skill in the art to use only those cells in which the amplified DNAs are stably inserted into chromosomal DNA." Lodish ¶ 134; *see also* Dolnick ¶ 29. However, as discussed in the preceding paragraph, amplified DNA II that is not stable in a host cell can confer resistance under selection pressure. The selection pressure scheme in claim 54 of the '216 patent may identify those cells that have amplified copies of DNA II; however, it does not necessarily distinguish between those cells in which the amplified DNA II is stable and those in which it is not. This was known in February 1980, as evidenced by the identification of cells that had amplified DNA residing in double minutes after selection.[38] Thus, it would not have been obvious to a person of skill in the art in February 1980 that the selection pressure of claim 54 of the '216 patent would distinguish host cells that had DNA II stably incorporated into the chromosomal DNA and those host cells that did not. Instead, it would distinguish those host cells that had a certain number of copies of DNA II and those that did not. In addition, it is important to emphasize that persons of skill in the art in February 1980 understood there was a potential distinction between the

[38] Ruddle, n. 26.

phenomenological observation of stability and the structural concept of integration within chromosomal DNA.

The plaintiffs' reports appear to assume that the mechanism of action of amplification that leads to the presence of amplified DNA in the '216 and '017 patents is one that requires both DNA I and DNA II to be present together in the chromosome. Kellems ¶¶ 43, 56; Lodish ¶ 137. The claims of the original Axel patents do not specify a mechanism of action for amplification that requires both DNA I and DNA II to be present in the chromosomal DNA. In addition, as of February 1980, it was not obvious that the mechanism of action for amplification of DNA I and DNA II required both DNA I and DNA II to be present on the chromosomes. To the contrary, researchers in the field admitted that they did not know the mechanism of action for amplification.[39]

In support of their attempt to import a mechanism of action into the claims of the original Axel patents, the plaintiffs' reports assert that experimental evidence from before February 1980 indicated that when the native DHFR gene is amplified in the chromosome, a larger DNA sequence than the DHFR gene was amplified.[40] However, this experimental evidence did not indicate that the amplified sequences around the DHFR gene were functional. Based on this experimental evidence, a person of skill in the art in February 1980 would not have considered it obvious that amplified DNA I and DNA II must be present together in the chromosome to amplify. In fact, even today this

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[39] Ruddle, n. 22.

[40] Nunberg, J. H., *et al.*, "Amplified Dihydrofolate Reductase Genes are Localized to a Homogeneously Staining Region of a Single Chromosome in a Methotrexate-resistant Chinese Hamster Ovary Cell Line," *Proc. Natl. Acad. Sci., USA* 75(11):5553-5556 (1978).

assertion has not been proven. There is continuing evidence that amplified DNA can exist unstably and outside of the chromosome.[41] In addition, even if amplified DNA units are integrated within the chromosome, there is experimental evidence suggesting that there can be deletions and ejections of genetic material previously incorporated in the chromosome. [42]

Finally, it is worth pointing out that plaintiffs' reports oversimplify the relationship between stability and incorporation. Plaintiffs' reports appear to assume that if DNA is incorporated into the chromosome, it is stable and will be passed on consistently to the host cell's daughter cells. Conversely, they maintain that if DNA is not incorporated into the chromosomes, it will not be stable and will not be passed on consistently to the host cell's daughter cells. However, the relationship between stability and incorporation within the chromosomal DNA is not totally clear. There is experimental evidence suggesting that standard chromosomal incorporation is not always required for stability.[43] In addition, there is experimental evidence at least suggesting

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[41] Schimke, R.T. *et al.*, "Chromosomal and Extrachromosomal Localization of Amplified Dihydrofolate Reductase Genes in Cultured Mammalian Cells," *Cold Spring Harb. Symp. Quant. Biol*, 45:785-797 (1981); *see* also, Ruddle, n. 26; Carroll, S. M. *et al.*, "Double Minute Chromosomes Can Be Produced from Precursors Derived from a Chromosomal Deletion," *Mol. Cell. Biol.* 8(4):1525-1533 (1988).

[42] *See generally,* Perucho *et al.*, "Genetic and Physical Linkage of Exogenous Sequences in Transformed Cells," *Cell* 22: 309-17 (1980).

[43] *See generally,* Hamkalo, B. A. *et al.*, "Ultrastructural Features of Minute Chromosomes in a Methotrexate-resistant Mouse 3T3 Cell Line," *Proc. Natl. Acad. Sci. USA* 82:1126-1130 (1985); *see also,* Kanda, T. *et al.*, "Mitotic Segregation of Viral and Cellular Acentric Extrachromosomal Molecules by Chromosome Tethering," *J. Cell. Sci.* 114(1):549-58 (2000).

that conditions can destabilize DNA incorporated into the chromosomes of a host cell.[44] Simply put, there is potentially a difference between integration in the chromosome and stability.

Just subsequent to February 1980, the understanding in the field about amplification and chromosomal integration greatly increased.[45] However, in February 1980, this information was not known. In February 1980 there was simply not enough known about amplification or about the location of amplified DNA to make it obvious that DNA I and DNA II (whether linked or unlinked when inserted) would both stably incorporate in the chromosomal DNA, or that if DNA I incorporated stably into the chromosomal DNA, that DNA II would do so as well. Because of the lack of precise analytical information to assess the process of amplification and the location of amplified DNA in host cells in February 1980, it would not have been obvious to a person of skill in the art that they could successfully create the host cell of claims 3, 5-14, and 16-19 of the '275 patent.

In addition, presently available scientific evidence indicates that DNA II will not necessarily stably incorporate into chromosomal DNA simply because it is linked to DNA I at one time, and/or because DNA I is stably incorporated into the chromosomal

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[44] Wurm, F.M. *et al.* "Effects of Methotrexate on Recombinant Sequences in Mammalian Cells," in *Gene Amplification in Mammalian Cells* 85-94 (Kellems, R.E. ed. 1993); Perucho *et al.*, "Genetic and Physical Linkage of Exogenous Sequences in Transformed Cells," *Cell* 22:309-17 (1980).

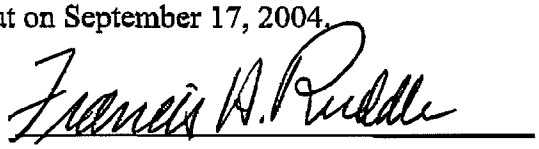
[45] *See generally,* Loyter, A. *et al.*, "Mechanisms of DNA Uptake By Mammalian Cells: Fate of Exogenously added DNA monitored by the Use of Fluorescent Dyes," *Proc. Natl. Acad. Sci. USA* 79:422-426 (1982).

DNA. Experimental data indicating the existence of unstable and extra-chromosomal amplified DNA, and the random and partial ejection of amplified DNA from stable incorporation in a chromosome, lead to the conclusion that depending on the type of cell and culture conditions, the typical location of DNA II could be chromosomal or extra-chromosomal.

V. Materials Relied On

Attached as Exhibit A is an additional list of materials that I have and/or may rely on in the future as part of my expert testimony.

Executed at New Haven, Connecticut on September 17, 2004.



Francis H. Ruddle